

The University of Chicago

MORPHOLOGICAL STUDY OF  
AGAVE LECHUGUILLA

A DISSERTATION SUBMITTED TO THE FACULTY  
OF THE DIVISION OF THE BIOLOGICAL SCIENCES  
IN CANDIDACY FOR THE DEGREE OF DOCTOR  
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1940

By  
ALVIN R. GROVE

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Reprinted from  
THE BOTANICAL GAZETTE  
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# MORPHOLOGICAL STUDY OF AGAVE LECHUGUILLA

## CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 534

ALVIN R. GROVE

(WITH THIRTY-EIGHT FIGURES)

### Introduction

STENAR (6) has compiled much of the literature of the Amaryllidaceae published previous to 1925. His discussion of the Agavoideae is brief, and much of his information is based on ERNST's work on *Fourcroya cubensis*. SCHAFFNER (3) and OSTERHOUT (2) have reported some work on pollen development, with special emphasis on the cytological investigation of microspore mother cell divisions and chromosome counts. SCHNARF (5) discusses in some detail the reproductive habits of some genera of the Amaryllidaceae, but statements concerning the genus *Agave* are meager and based again in large part on ERNST's work on *Fourcroya*. SCHLIMBACH (4), in discussing the Amaryllidaceae, makes general reference to the family but does not present details concerning *Agave*. GOEBEL (1) makes several short references to this genus, speaking in particular of its hollow style and the development of a multilayered endothecium in *A. americana*.

The research reported here includes studies of the origin and development of the floral organs and their relation to the axis and bracts which become involved in such development; the vascular anatomy of the flower; the development of the ovule; megasporogenesis and megagametogenesis; and the development of the anther. No effort has been made to count the chromosomes or to make a cytological investigation of the nuclear divisions. The present investigation started with actual flower formation, but much growth of the flowering axis has already preceded this stage. Elongation of the axis from the center of a basal rosette of foliar leaves is the first indication of flowering. This stalk elongates rapidly and may reach a height of many feet before recognizable flowers appear. The material studied was collected from the tip of this axis after its elongation had taken place.

### Material and methods

Material of *Agave lechuguilla* Torr. was collected in the field near El Paso, Texas, May 7, 1939, and June 1, 1940, and also at Alpine, Texas, May 5, 1939. Flowers and developing fruits were dissected from healthy plants and were killed and fixed in Sax's modification of Navashin's solution. The material was separated into approximately twenty age groups, from tips to oldest fruits, and later sectioning was done on random samples from these groups. Treatment was according

to the paraffin method, and sectioning was at  $3-15\ \mu$ . Flemming's triple stain and light green and safranin were employed. At the time that material was fixed for sectioning, other material was killed in formalin-acetic-alcohol and used for gross study.

### Observations

#### ORIGIN AND DEVELOPMENT OF FLOWER

The first evidence of floral development is the appearance of a single branch primordium in the axil of each bract, referred to here as the outer bract, diverging from the main axis of the plant (figs. 1, 7). Before appreciable elongation of this branch primordium has taken place, two bracts, referred to here as the adaxial flower bracts, arise near its base and toward the axis. As development of these bracts takes place, their bases elongate conjointly and at first only their tips are separate. Later, after the flowers themselves are well developed, the conjoint base splits so that apparently two completely separate bracts are present, one on the adaxial side of each flower branch. Before any elongation of the first branch primordium has taken place a second one arises in the axil of the bract on the first branch primordium. In a cross section of the young flowering axis these two primordia lie inside the outer bract and outside the conjoint base of the two developing adaxial bracts (fig. 7). The origin of the first and second branch primordia and the two adaxial bracts can be determined from cross sections in which the bases of all four structures are common. Later, as elongation takes place, each can be seen as a separate structure near its tip. The first and second branch primordia are completely covered with an epidermis, as are both adaxial bracts. In addition to the structures already mentioned, each flower primordium bears a bract on its outer face, the abaxial bract. These bracts develop later than the adaxial bracts, and flowers may or may not develop in their axils. This development usually leads to the formation of two flowers, one differentiated at the tip of the first branch primordium and one at the tip of the second. Not always, however, does development stop with two flowers, and cases can be found where three or even four ultimately develop. Such a branch system of flower origin would seem the normal behavior for the plant and accounts for the origin and variation in number of flowers present.

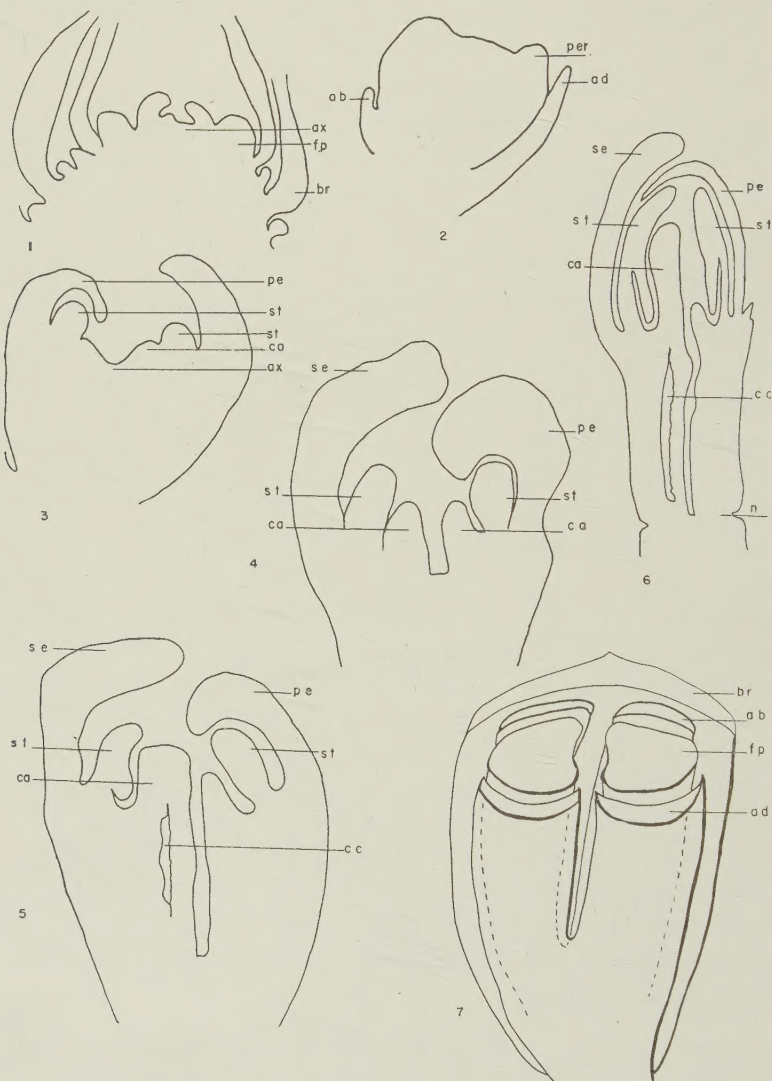
#### PERIANTH AND SEPALS

The perianth arises first as a ring on the periphery of the flower primordia. Not long after, the three sepals arise from this as separate primordia. At this time there is much lateral growth of the end of the branch, which causes it to widen and flatten (fig. 2). Subsequent elongation of the sepal primordia (figs. 3-6) leads to three elongated and flattened sepals which are widened at their base and which in the mature flower overlap the petals to some extent.



## PETALS

The petal primordia, three in number, arise next in order and lie just inside the ring of sepal primordia and alternate with them (fig. 3). They eventually elongate



FIGS. 1-7.—Figs. 1-6, successive development of floral parts. Fig. 7, reconstruction showing relation of bracts to flower primordia; axis removed. (*ax*, axis; *fp*, flower primordia; *br*, outer bract; *ab*, abaxial bract; *se*, sepal; *pe*, petal; *ca*, carpel; *st*, stamen; *cc*, carpellary cavity; *n*, node.)

and are sepaloid in appearance (figs. 4-6), contain chlorophyll, and are not showy. In shape they are similar to the sepals and approximate them in size. In the mature flower their edges are overlapped by alternating edges of the sepals.

## STAMENS

Two rings of three stamens each are next in origin. The outer ring of three lies opposite the three sepals and the inner ring of three within and opposite the three petals. The primordia arise as more or less dome-shaped protuberances, and all six stamens develop simultaneously in two cycles (fig. 3). At the time the stamens originate the sepals are already well developed and the petals are rapidly elongating. In later stages of development the anther arises as a terminal expansion of the stamen. Its growth is parallel to the filament, and in the mature condition the filament is attached at about the center of the elongated anther, being versatile. When the flower opens at time of pollination the anther flips up, its median attachment to the filament operating as a hinge (fig. 6).

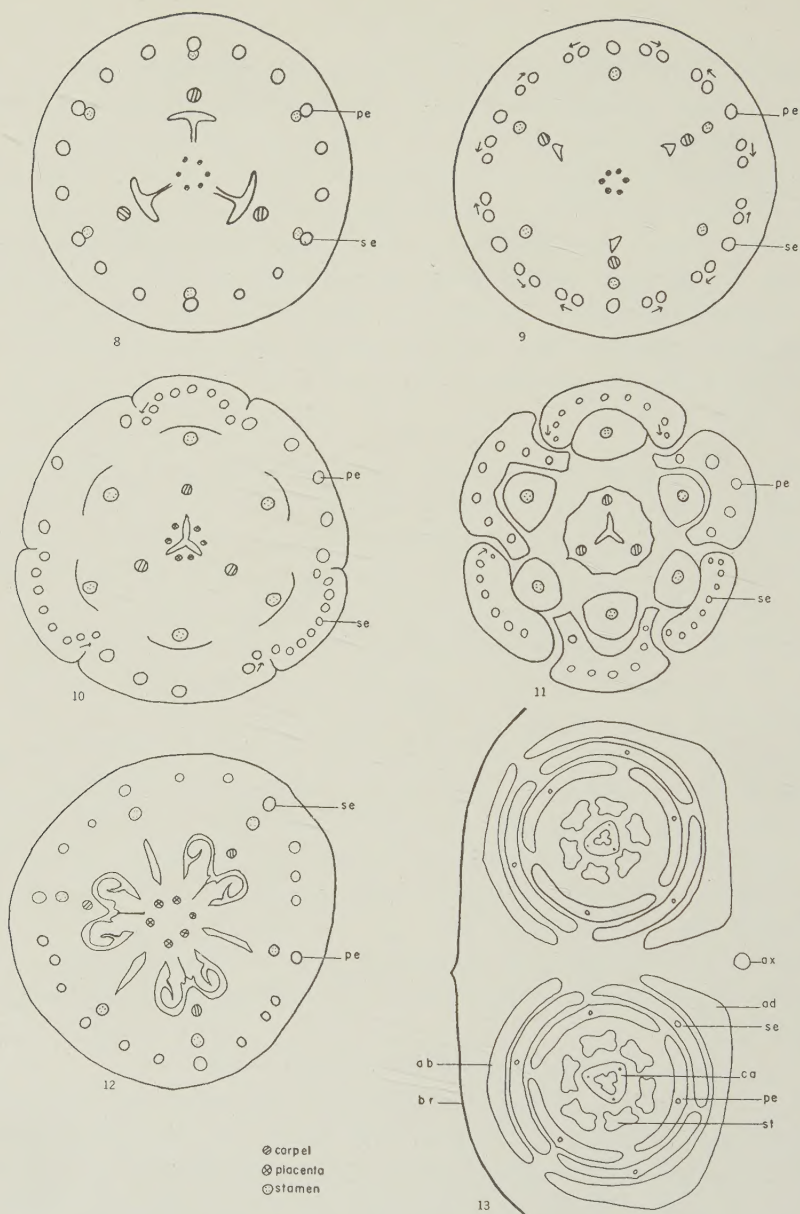
## CARPELS

The three carpel primordia arise last and opposite the sepals, and, like the stamens, they are at first more or less dome shaped (fig. 3). At this stage in ontogeny the growth of the end of the axis is inhibited, but all the conjoint flower parts continue to elongate and enlarge above it, so that in the young flower a canal is left in the center of the developing ovary. However, the conjoint development of the three carpels forms a common central tissue which completely fills this central canal in the mature flower (fig. 12) and leads to the formation of a compound ovary with three separate and completely inclosed carpellary cavities. Zonal elongation of the conjoint bases of the three carpels completes the formation of the compound pistil. The tips of the three carpels continue above the ovary as a compound style, which is three lobed and hollow. As zonal elongation of the three carpels takes place, zonal elongation of the undiverged bases of the sepals, petals, and stamens also occurs. This conjoint tissue completely surrounds and is indistinguishable from the carpels (fig. 12).

The general development just described is one of acropetal succession, starting with sepals and progressing inward toward the center of the flower through petal, stamen, and carpel.

## VASCULAR ANATOMY OF FLOWER

A node at the bottom of the ovary makes it difficult to trace the vascular supply of the flower down through the flower branch (fig. 6). In a very young flower, before differentiation of the majority of the procambial strands, it is possible to trace a certain amount of vascular tissue through the node, but since all traces are not differentiated, the lack of obvious nodal ramifications complicates the interpretation rather than simplifies it. For this reason no attempt has been made in the drawings to trace the vascular supply through the node; only the bundles above it after vascular tissue differentiation has taken place have been recorded (figs. 8-13). In no instance have drawings been included at the tips of sepals, petals, etc.



FIGS. 8-13.—Figs. 8-11, successive cross sections at representative levels. Fig. 12, cross section of older ovary showing relation of ovules. Fig. 13, diagrammatic cross section of two flowers, showing orientation of floral members. (se, sepal; pe, petal; ax, axis; ad, adaxial bract; ab, abaxial bract; br, outer bract; ca, carpel; st, stamen.)



At the base of the ovary there is visible a median bundle for each sepal and each petal. In addition there are twelve lateral bundles, one on either side of each median sepal and petal bundle. On the inner side of each median sepal and petal bundle is a stamen bundle. In most cases at this level this bundle is so closely appressed that it is difficult to distinguish it. Three dorsal carpel bundles are present at this level and remain unchanged throughout the upper extent of the flower, passing into the style at the top of the ovary. Six placental bundles are present, but at this stage show little differentiation (fig. 8).

Examination of cross sections of the flower at higher levels shows that the median bundle of each sepal passes into the sepal without dividing. Each lateral bundle is divided to give rise to one toward the edge of the sepal. The bundles formed by this first division divide, and the derived bundles here divide once. This leads to the usual condition of nine bundles present in the sepal after its divergence at the top of the hypanthium (fig. 11). The petal bundles show the same kind of development, except that two divergences usually occur and seven bundles rather than nine is the usual number present in the petal (fig. 11). The stamen bundles, as already mentioned, lie inside of and are tightly appressed to each median sepal and petal bundle (fig. 8). At the top of the hypanthium the stamen bundles pass directly up into the stamens (fig. 11).

The vascular plan above the node is a simple one, and indicates that the hypanthium consists of the undiverged bases of sepals, petals, and stamens, as shown by their vascular anatomy.

#### OVULE DEVELOPMENT

The nucelli arise from the lateral edges of each of the three carpels. As growth continues the nucellus elongates, and with growth more rapid on the outer face the nucellus gradually turns through an arc of nearly  $180^\circ$  to become anatropous. Two integuments are developed from the base of the nucellus and ultimately overgrow its end to form the micropyle. The outer integument is fully developed only on the outer face of the ovule and is present only as a ring on the funicular side.

Before any appreciable curvature of the nucellus, and previous to the origin of the inner integument, the archesporial cell is differentiated. The megaspore stages are numerous when the nucellus has passed through about a  $90^\circ$  arc, and one integument is developed—with the second starting in some instances. Megagametophyte stages occur when the ovule has assumed its complete anatropous position and both inner and outer integuments are well developed.

#### MEGASPOROGENESIS

A single hypodermal cell enlarges from the sporophytic tissue of the nucellus. It can readily be identified by its position, immediately beneath the epidermis; its increased size and denser cytoplasm; and its enlarged nucleus. It is differentiated

before the appearance of the inner integument and before any appreciable growth of the nucellus has taken place (fig. 14). The first and only division of the archesporial cell is periclinal, resulting in the primary sporogenous cell on the inside and the primary parietal cell on the outside (fig. 15). The primary parietal cell is divided in turn by an anticlinal division (fig. 16) and eventually gives rise to a single parietal layer (figs. 17-20). The parietal tissue persists until the megagametophyte starts to enlarge, at which time it is crushed.

The primary sporogenous cell enlarges directly into the megaspore mother cell (fig. 17). Two divisions of this cell and its derivatives follow in rapid succession, and a linear tetrad of four megaspores is formed (figs. 18, 19). Not until both divisions are completed is there evidence of wall formation, but following the second division walls come in to form four cells (fig. 20). Although chromosome counts were not made, it is assumed that these two divisions involve reduction of the chromosomes from the diploid to the haploid number. Of the linear tetrad so produced the megaspore toward the chalazal end of the ovule survives and the remaining three disintegrate (fig. 20). This results in an enlarging uninucleate cell which becomes directly the young megagametophyte (fig. 23).

#### VARIATION IN MEGASPORE ARRANGEMENT

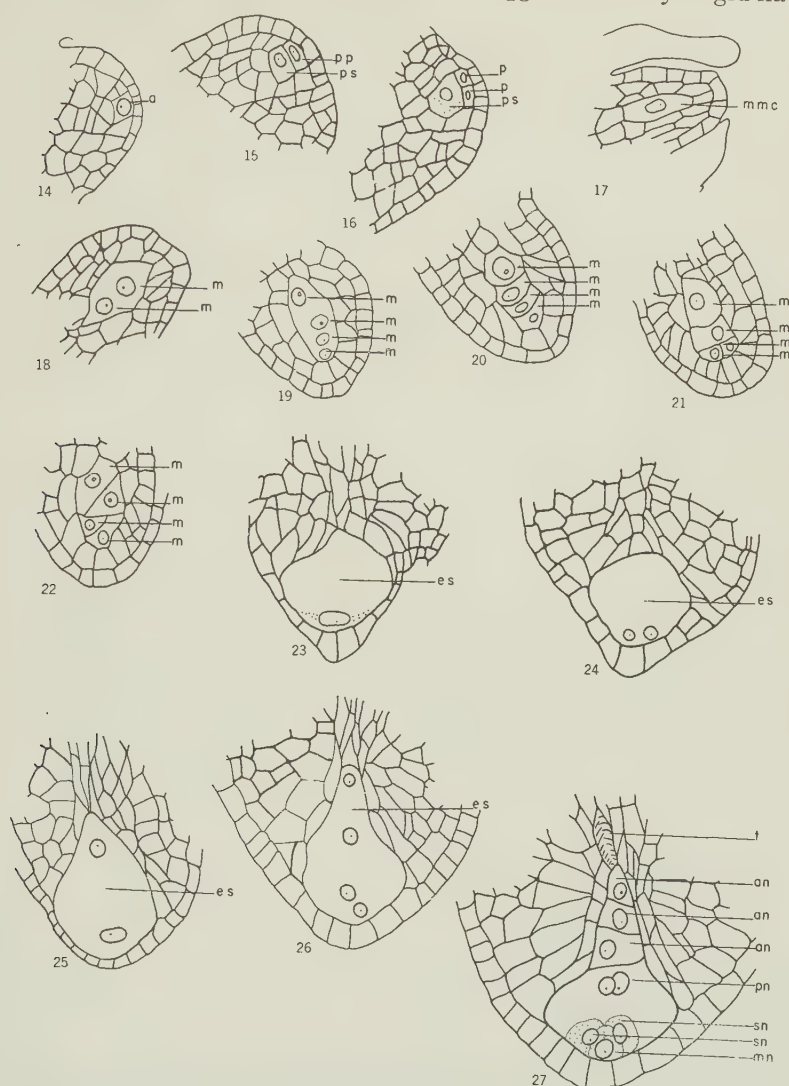
In addition to the usual linear tetrad arrangement of the four megaspores, two variations were noted rather commonly. One variation is essentially the same as the linear tetrad, except that the walls between the megaspores come in at angles to one another instead of parallel (fig. 22). A second variation shows the wall between the two micropylar nuclei at right angles to the wall between the other two toward the chalazal end (fig. 21). This is similar to a condition already recorded for *Haemanthus katharinae* and *Bomarea caldasii* (6).

#### MEGAGAMETOPHYTE DEVELOPMENT

The single nucleus of the enlarging young megagametophyte is located near the micropylar end (fig. 23). It divides to give rise to two nuclei (fig. 24) and one nucleus, then migrates toward the chalazal end (fig. 25). The cytoplasm between these two nuclei, one the primary chalazal and the other the primary micropylar, becomes highly vacuolated as enlargement occurs. Each of these two primary nuclei divides to give rise to a four-nucleate megagametophyte (fig. 26). Division of the primary micropylar nucleus precedes that of the primary chalazal nucleus. Each of the four nuclei now present undergoes one more division, giving rise to eight. Of the four at the micropylar end of the megagametophyte, one develops as the egg, two as synergids, and one as a polar nucleus. Three of the four nuclei at the chalazal end become nuclei antipodal cells arranged in linear manner and one a polar nucleus. The two polar nuclei migrate toward the center of the megagame-



tophyte and together make the primary endosperm nucleus, fusion not taking place until fertilization or soon thereafter. The egg and each synergid have a defi-



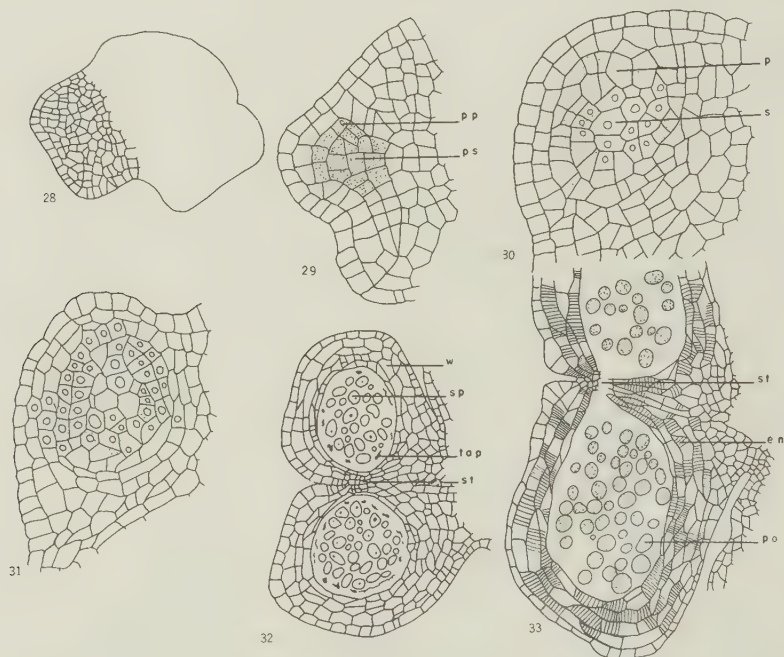
FIGS. 14-27.—Megasporogenesis and megagametogenesis. (*a*, archesporial cell; *pp*, primary parietal; *ps*, primary sporogenous; *p*, parietal; *mmc*, megaspore mother cell; *m*, megaspore; *es*, embryo sac; *t*, tracheid; *an*, antipodal nucleus; *pn*, polar nuclei; *sn*, synergid; *mn*, megagamete nucleus.)

nite wall and a fairly large amount of cytoplasm. The cell containing the two primary polar nuclei is much larger than any of the others (fig. 27). The cytoplasm of the two synergid cells cups slightly around the egg cell, which takes a central position between the two synergid cells.

Instead of the vascular tissue ending at the base of the chalaza, tracheids are often differentiated in the tissue between the chalaza and the megagametophyte itself. Apparently there are only tracheids and there is no indication that phloem elements are present also. This condition is rare in plants (fig. 27).

#### MICROSPORES AND MICROGAMETOPHYTE

The anther, when mature, is terminal and versatile. As its development proceeds, four distinct lobes are formed. At first, all the cells of these lobes, exclusive of the epidermis, are similar in size, in density of cytoplasm, and in general appearance (fig. 28).

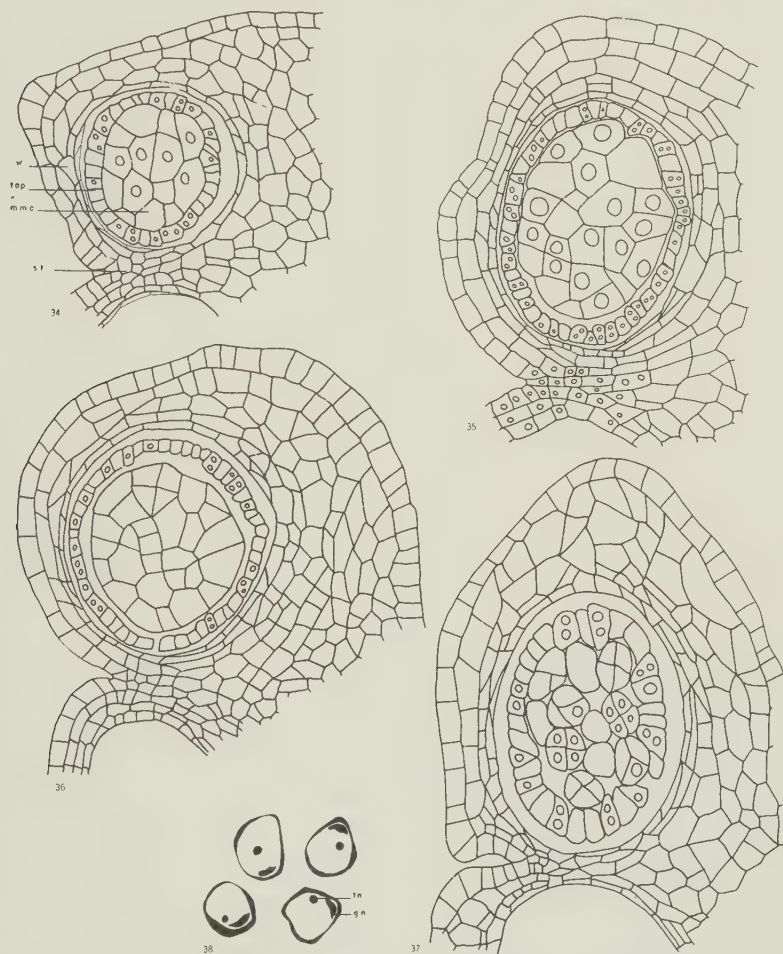


FIGS. 28-33.—Anther development. Figs. 28-31, early development of parietal and sporogenous tissue. Fig. 32, microspore separation. Fig. 33, mature anther. (*ps*, primary sporogenous; *pp*, primary parietal; *w*, wall; *st*, stomium; *en*, endothecium; *sp*, spores; *po*, pollen; *tap*, tapetum; *s*, sporogenous; *p*, parietal.)

The archesporium differentiates as four groups of cells from hypodermal tissue, one in each lobe of the anther. The archesporium is not easily distinguished at first, but by the time of periclinal division of these cells in the formation of primary parietal and primary sporogenous tissue it is easily distinguished (fig. 29). The primary parietal tissue thus formed undergoes several other periclinal and random anticlinal divisions in the formation of wall tissue which surrounds the more or less centrally located sporogenous mass (figs. 29-31). The innermost layer of parietal cells differentiates as tapetum. The cells of the tapetal layer are small at first but



gradually enlarge, and by the time microspore mother cells are recognized many of the tapetal cells are binucleate. Although the tapetum is fully formed by the time microspore mother cells are differentiated, further enlargement of the tapetal



FIGS. 34-38.—Anther development. Fig. 34, microspore mother cells. Fig. 35, at time of first reduction division. Fig. 36, at time of second reduction division. Fig. 37, separation of spores. Fig. 38, mature pollen. (*mmc*, microspore mother cell; *tap*, tapetum; *w*, wall; *st*, stomium; *tn*, tube nucleus; *gn*, generative nucleus.)

cells does take place and its greatest development is simultaneous with the formation of microspores. From this period on the tapetum disappears and the microspores enlarge and develop. In some instances at least, disintegration of the tapetum supplies a nutritive mass which surrounds many of the newly separated spores. The tapetum and its remnants continue for a long time during spore de-

velopment, but when mature pollen grains are found it has entirely disappeared (figs. 34-37).

The remaining wall cells derived from the parietal tissue stay more or less intact, although there may be some disintegration of the inner layer as the tapetum enlarges. When the anther matures the remaining wall tissue is characterized by the formation of a well-defined endothecium, which involves all but the epidermal layer of wall tissue (fig. 33).

The primary sporogenous tissue is formed by division of the archesporium. These primary sporogenous cells may undergo one or more divisions and the resulting cells enlarge and function as microspore mother cells (figs. 30, 34). The microspore mother cell undergoes two successive divisions, the reduction divisions, and wall formation follows each immediately, forming four microspores (figs. 34-36). Following the second division the microspores separate, usually in groups of four cells, following the pattern of the original microspore mother cells; but these groups soon separate, the individual spores becoming independent. They are at first thin walled and uninucleate and may in part be surrounded with a cytoplasmic mass from the disintegrating tapetal cells (figs. 32, 37). As the microspores mature their wall continues to thicken, and division of the nucleus results in the formation of a two-nucleate microgametophyte. The tube cell is large and occupies the greater part of the pollen grain. In cross sections of mature pollen grains one thin place is always recognized in the wall surface surrounding the tube cell.

The generative nucleus is vermiform and its cell is considerably smaller and less regular in outline than the tube cell (fig. 38). Stomium structure is traced in figures 28-37. As the anther matures, with pollen grains and endothecia differentiation, the stomium breaks with little decomposition and the locular spaces of the two adjoining pollen groups become one (fig. 33).

### Summary

1. Origin of the floral organs of *Agave lechuguilla* is acropetal, sepals appearing first and carpels last.
2. The flower is epigynous. Its vascular anatomy is simple, being a direct divergence of vascular bundles into the separate organs. All vascular bundles ending in either sepal or petal structures are derived from bundles differentiated early in connection with these floral parts.
3. The ovule is anatropous and has two integuments. Placentation is axial.
4. Megasporogenesis starts with differentiation of a single hypodermal archesporial cell and ends with formation of a tetrad of megaspores arranged usually in linear fashion. Two variations in megaspore arrangement were noted.



5. Periclinal division of the archesporial cell gives rise to a primary parietal cell and to a single primary sporogenous cell which enlarges and becomes the megaspore mother cell. Development of the megagametophyte follows the so-called normal type.

6. A single parietal layer is present in the ovule, disintegrating when the megagametophyte enlarges.

7. The vascular tissue of the ovule does not stop at the base of the chalaza but tracheids penetrate the nucellus to the megagametophyte.

8. Anther development follows the common method. The archesporium consists of a group of cells. Periclinal divisions give rise to the primary parietal and primary sporogenous cells. Continued periclinal and anticlinal divisions of the primary parietal cells form the wall tissue. The sporogenous cells enlarge and function as microspore mother cells. There are two successive divisions of the microspore mother cells, resulting in four microspores.

9. The tapetum is derived from the innermost layer of the parietal tissue.

10. The mature anther shows well-defined endothecia three or four cells in thickness and disintegration of the stomium.

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